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Effects of recombinant human interleukin-12 on eosinophils, airway hyper-responsiveness, and the late asthmatic response

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Summary

Background Interleukin-12 (IL-12) is a macrophage-derived cytokine that modulates T lymphocyte responses and has the capacity to suppress allergic and eosinophilic inflammation.

Methods We carried out a double-blind, randomised, parallel group clinical study, in which patients with mild allergic asthma were given subcutaneous recombinant human IL-12 at increasing weekly injections of 0·1, 0·25, 0·5 μg/kg (n=19), or placebo (n=20). We compared responses to inhaled allergen challenge 24 h before the first injection and 24 h after the final injection. Airways hyper-responsiveness and concentrations of peripheral blood eosinophils and sputum eosinophils were also assessed.

Findings IL-12 caused a significant decrease from baseline in the main peripheral blood eosinophil count 24 h after the fourth injection compared with placebo (p=0·0001). Sputum eosinophils were also significantly decreased 24 h after allergen challenge when treated with IL-12 compared with placebo (p=0·024). IL-12 caused a non-significant trend towards improvement in airway hyper-responsiveness to histamine, but had no significant effect on the late asthmatic reaction after inhaled allergen challenge. After administration of IL-12, four of 19 patients withdrew prematurely; two with cardiac arrhythmias, one with abnormal liver function, and a single patient with severe flu-like symptoms.

Interpretation We have shown that IL-12 lowers numbers of blood and sputum eosinophils, but without any significant effects on airway hyper-responsiveness or the late asthmatic reaction. This questions the role of eosinophils in mediating these reactions, and has important implications for development of new anti-inflammatory treatments.

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Introduction

Allergic asthma is characterised by inflammation involving the accumulation of eosinophils and mononuclear cells in the airways. This inflammation is caused by a defect in immune regulation involving T helper lymphocytes, with an increase in Th2 lymphocytes and a compensatory decrease in Th1 lymphocytes.[1] Th2 cells generate cytokines that are responsible for the pathogenesis of asthma; these include interleukin-5 (IL-5) which leads to eosinophilia, as well as IL-4 and IL-13 which contribute to the production of IgE.[2,3]

IL-12 is a key cytokine involved in regulating the balance between Th1 and Th2 cells.[4,5] Cord blood from infants genetically predisposed to atopy is deficient in interferon-γ production,[6] a defect attributed to impaired IL-12 responses.[7] Decreased concentrations of IL-12 are reported in blood from adults with allergic asthma,[8] thus predisposing to excess IgE synthesis[9] and IL-5 production.[10] Furthermore, IL-12 inhibits airway hyper-responsiveness and airway eosinophilia after antigen challenge in several animals with allergen sensitisation.[11,12] Hence, IL-12 would seem to be a logical treatment for human allergic asthma, especially because it has the potential to interfere with the natural history of allergic disease.

Inhaled allergen challenge in people with asthma has been used extensively to study the pathogenesis of asthma and in the clinical testing of anti-inflammatory treatments for asthma. In susceptible individuals there is an early asthmatic response within the first 2 h, followed by a late asthmatic response at 4 to 10 h. The late asthmatic reaction is associated with blood and sputum esoinophilia and airway hyper-responsiveness.[13,14] We aimed to assess the effects of a course of four subcutaneous injections of increasing concentrations of recombinant human IL-12 on blood and sputum eosinophils and airway responses to histamine and allergen in people with mild asthma.

Methods

Study population

We studied 39 adults with allergic asthma (table 1). The inclusion criteria were a history of mild asthma requiring inhaled β_2-agonists only, forced expiratory volume in 1 s (FEV_1) 70% or more of predicted normal, a positive allergen skin-prick test, no use of corticosteroids for the previous month, no tobacco smoking within the past 5 years, provocative concentration of histamine ≤8 mg/mL, and early and late responses to inhaled allergen. Exclusion criteria were symptoms of an upper-respiratory tract infection in the previous 2 weeks, allergy to grass pollen during the pollen season, and a history of a medical illness other than asthma. All participating patients signed consent forms approved by the local ethics committees, and were admitted to specialist units for treatment and clinical monitoring.

Characteristics	IL-12 (n=19)	Placebo (n=20)
Sex		
Male	12	15
Female	7	5
Age (years)	25·8 (7·5)	26·4 (5·3)
Height (cm)	174·2 (10·1)	174·3 (8·5)
Weight (kg)	76·1 (14·8)	74·4 (11·7)
Serum IgE (fU/mL)	440 (433)	241 (149)
Serum eosinophil cationic protein (IU/mL)	36·5 (34·8)	28·3 (14·9)
Daily symptom score*	0·5 (0·41)	0·49 (0·38)
Morning peak expiratory flow (L/min)	475 (88)	481 (82)
Daily albuterol actuations	0·8 (1·1)	1·9 (2·8)

Values are mean (SD). *Daily symptom score: 0=absent, 1=mild, 2=moderate, 3=severe.

Table 1: **Baseline characteristics of patients**

Study design

Recombinant IL-12 is a recombinant DNA-derived protein produced by a suspension of Chinese hamster ovary cells grown in a medium without bovine serum. Ro-24-7472 was purified from the cell supernatant by four chromatographic and two viral inactivation steps and formulated for subcutaneous injections. Final purity was greater than 99%, and endotoxin was not detectable. At screening, patients underwent a histamine challenge, a skin-prick test (house dust mite, timothy grass pollen, cat hair, and histamine) and a baseline incremental allergen challenge. We administered recombinant human IL-12 (increasing doses of 0·1, 0·25, 0·5, 0·5 μg/kg) to 19 patients and placebo (sodium chloride 0·9%) to 20 patients by subcutaneous injection once a week for 4 weeks (figure 1). At each visit, spirometry was done and blood sampled for antibodies to IL-12, IgE, haematology, and liver enzymes. Bolus inhaled allergen challenge was done 24 h before the first injection and 24 h after the fourth injection, with FEV_1 measurement at frequent intervals for up to 10 h after inhalation of allergen. We measured histamine reactivity before each bolus allergen challenge and 24 h later when sputum had been induced. We did a follow-up visit 4 weeks after the final injection.

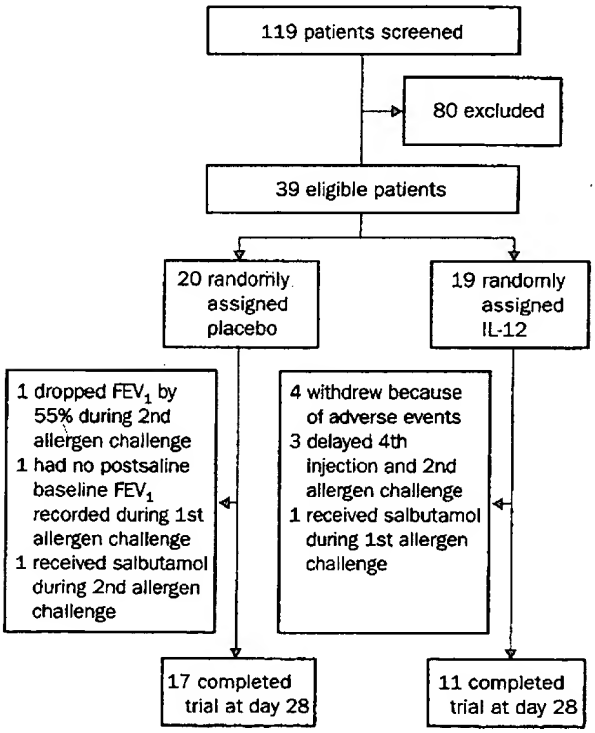

Figure 1: **Trial profile**

Bronchial challenge tests

Airways reactivity to histamine was measured by inhalation of doubling doses of histamine (0·03–32 mg/mL) and the provocative concentration of histamine causing a 20% decrease in FEV_1 was calculated. Inhaled allergen challenges were done according to previously described methods.[15] The allergen to which the patient was most responsive to in the skin prick test was freeze-dried, and diluted in 0·9% saline to make up doubling doses of allergen ranging from 250 to 32 000 standardised quality units (SQU/mL). In the screening incremental allergen challenge, FEV_1 was measured after inhalation of saline and then after increasing concentrations of allergen, but after the early phase of the allergen challenge (ie, ≥15% drop in FEV_1 from baseline) FEV_1 was measured at half-hourly intervals for up to 10 h. A late reaction was defined as a drop in FEV_1 of 15% or more on 3 or more occasions between 4 and 10 h after inhalation of allergen. For bolus allergen challenges, allergen was administered as a bolus dose that was equal to the sum of the incremental doses inhaled by the patient in the screening allergen challenge.

Induced sputum

We induced sputum and processed it using a standard protocol;[16] patients inhaled hypertonic saline (4·5%) from a nebuliser for three periods of 5 min and were encouraged to expectorate sputum immediately after each inhalation period. The sputum sample was placed in a Petri dish and the solid portion (sputum plugs) was separated from the saliva and weighed, before adding four times the volume of dithiothreitol (0·1%) and doing liquefaction, filtration, and centrifugation. The cell pellet was used to form cytospins, and samples of the supernatant were stored at −20°C.

Statistical analyses

All results are reported as mean (95% CI). For the primary variable of late asthmatic reaction, the area under the curve for the percentage change in FEV_1 from baseline at 4 to 10 h after allergen challenge was calcualted, and the decrease from the late asthmatic reaction before treatment and after was analysed. We did an analysis of covariance (ANCOVA) for the late asthmatic response at week 4, with treatment as the factor and the late asthmatic response at week 1 as the covariate. We did between group comparisons of histamine reactivity with *t* tests. Group means of peripheral blood eosinophil concentrations were compared 24 h after the first and last injections of IL-12 or placebo using an ANCOVA with treatment as factor and blood eosinophils at baseline as covariate. Paired *t* tests were applied for within group comparisons of peripheral blood eosinophils at baseline and day 23. Sputum eosinophil counts were related to non-squamous cell counts to keep variability to a minimum. An explorative ANCOVA with the percentage of eosinophils at week 4 (day 24, postallergen challenge) as target variable, treatment as factor, and the percentage of eosinophils at baseline as covariate, was done. The treatment effects were assessed by the means adjusted to the total mean of the baseline eosinophil percentages at baseline. Since many patients with mild asthma were recruited, a log-transformation of the sputum eosinophil percentages, as is often used in the case of percentages to improve the approximation to a normal distribution, was not applied. If applied, this transformation would have given these patients with small sputum eosinophil percentages more weight in the analysis than when using the raw values.

	IL-12	Placebo	Difference	p-value by ANCOVA
Blood eosinophils (×10⁹/L)				
Day 2*	0·214 (0·119–0·311)	0·407 (0·328–0·487)	0·176 (−0·300 to −0·052)	covariate: p=0·146 treatment: p=0·007
Day 23†	0·041 (−0·017 to 0·100)	0·265 (0·218–0·311)	−0·212 (−0·287 to −0·138)	covariate: p=0·147 treatment: p=0·0001
Sputum eosinophils (%)				
Day 1‡	18·6 (7·8–29·5)	22·0 (10·0–34·0)	−8·9 (−16·5 to −1·3)	covariate: p=0·0001
Day 24§	8·3 (4·5–12·1)	18·7 (7·4–29·9)		treatment: p=0·024
Late asthmatic reaction (area under curve)				
Day −1‖	131·9 (93·7–170·2)	174·5 (139·5–209·5)	6·5 (−38·1 to 51·1)	covariate: p=0·0001
Day 23†	112·9 (67·1–158·7)	140·3 (100·8–179·8)		treatment: p=0·77
% reduction of reaction	0·115 (−0·161 to 0·391)	0·184 (−0·028 to 0·395)	−0·069 (−0·397 to 0·260)	p=0·67
Histamine PC_{20} (mg/mL)				t-test:
Day 1‖	0·75 (0·42–1·08)	0·57 (0·35–0·80)	0·18 (−0·19 to 0·54)	p=0·33
Day 1‡	0·73 (0·25–1·21)	0·57 (0·28–0·86)	0·16 (−0·34 to 0·65)	p=0·52
Day 23†	1·38 (0·37–2·40)	1·59 (0·64–2·54)	−0·21 (−1·61 to 1·20)	p=0·76
Day 24§	0·82 (0·25–1·40)	0·63 (0·36–0·90)	0·19 (−0·35 to 0·73)	p=0·47

Values shown as mean (95% CI). *24 h after 1st injection. †Before 2nd allergen challenge, 24 h after 4th injection. ‡24 h after 1st allergen challenge, before 1st injection. §24 h after 2nd allergen challenge, 48 h after 4th injection. ‖Before 1st allergen challenge and injection. PC_{20}=provocative concentration.

Table 2: **Effects of IL-12 on biomarker responses to allergen challenge**

Results

After IL-12 treatment, an influenza-like syndrome consisting of headache, fever, and limb myalgia was seen in most participants. Four such patients were withdrawn from the study. One patient had raised serum transaminase concentrations (more than five times the upper range of normal, although this was transient and reversed without any striking clinical symptoms). Another patient had a symptomless run of six consecutive premature ventricular ectopic beats, and echocardiography subsequently showed that this patient had mild mitral valve prolapse. One patient withdrew from the study because of an episode of syncope preceded by palpitations that recovered spontaneously, and nausea and excessive flu-like symptoms.

The late asthmatic reaction was the area under the curve for bronchoconstriction (percentage change of FEV_1 from baseline) at 4 to 10 h after allergen challenge (table 2). The reaction was not substantially changed after treatment, with minor mean reductions of the area under the curve in the late asthmatic reaction after placebo or IL-12. The mean difference was not significant.

Airway hyper-responsiveness to histamine was measured before and 24 h after each allergen challenge. Although there was a trend towards some improvement after allergen challenge with IL-12 compared with placebo (table 2), it was not significant.

To analyse the effects of IL-12 on blood eosinophils after the first and last dose of treatment, between group comparisons were done on day 2 and day 23 (24 h after the first and last doses; table 2, figure 2). At day 2 after the first dose of the study treatment, there was already a significant difference in concentrations of blood eosinophils between the placebo and IL-12 groups, indicating a rapid onset of action. At day 23, 1 day after the last dose, there was again a significant difference between the groups. At day 25, 48 h after the second allergen challenge, blood eosinophils were increased in both groups, however, the mean blood eosinophil counts for the IL-12 group remained lower than that of the placebo group.

48 h after the first allergen challenge and 24 h after the first injection (day 2), patients receiving IL-12 showed no increase in blood eosinophils in contrast with the placebo group. However, 48 h after the second allergen challenge and 72 h after the fourth injection (day 25), blood eosinophil concentrations increased in both groups. There was a decrease in blood eosinophils after the first allergen challenge, but an increase in blood eosinophils after the second allergen challenge. This finding could be explained by the timing of IL-12 treatment; the first dose of IL-12 was given 24 h before blood eosinophil estimation on day 2, whereas the fourth dose was given 72 h before blood eosinophil estimation on day 25. At the follow-up visit 4 weeks after the last injection, blood eosinophil counts had returned to baseline in both groups. We analysed baseline blood samples before both histamine and allergen challenges. There were no significant changes seen in other leucocyte populations after treatment with placebo or IL-12.

In the patients who completed the trial, IL-12 resulted

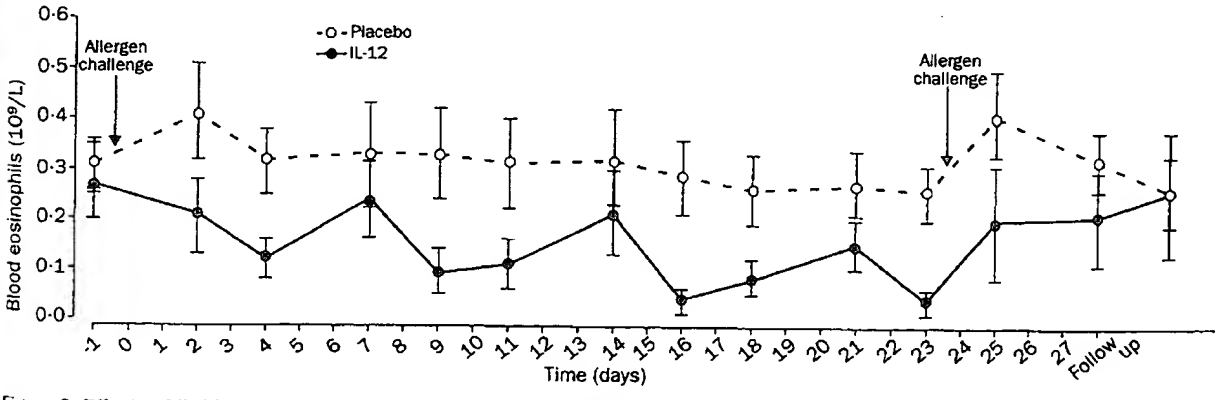

Figure 2: **Effects of IL-12 and placebo on blood eosinophils in people with asthma**
Injections given on days 1, 8, 15, and 22.

in a significant decrease in the number of circulating eosinophils from mean $0{\cdot}27\times10^9$/L (95% CI 0·19–0·34) 24 h before the first injection to $0{\cdot}04\times10^9$/L (−0·02 to 0·10) 24 h after the fourth injection (day 23; paired *t* test, p=0·0004; figure 2). In the intention-to-treat analysis, IL-12 was also shown to cause a significant decrease in the number of blood eosinophils from $0{\cdot}42\times10^9$/L (0·29–0·54) to $0{\cdot}09\times10^9$/L (0·02–0·16; paired *t* test, p=0·0001). IL-12 treatment decreased blood eosinophil numbers within 24 h, with numbers gradually increasing at 4 to 7 days after injection.

After IL-12, the number of blood eosinophils was maintained below baseline throughout the treatment period (day 2 to day 23). With each sequential injection of IL-12 there was a progressive decrease in the eosinophil count, when comparing eosinophil concentrations at 24 h and 6 days after consecutive injections. In the placebo group completing the protocol, the blood eosinophil count changed slightly from $0{\cdot}30\times10^9$/L (0·25–0·34) to $0{\cdot}27\times10^9$/L (0·22–0·31) and rose again 24 h after allergen challenge.

An ANCOVA with the percentage of eosinophils at week 4 (day 24 postallergen challenge) as the target variable, treatment as factor, and the percentage of eosinophils at baseline as covariate, revealed a significant difference between the treatment groups (p=0·024). The estimated treatment effect on eosinophil percentages at week 4 was −8·9% (adjusted for the total mean of eosinophil percentages at baseline; table 2). In this analysis, all patients with valid pairs (baseline and day 24) of sputum eosinophils have been included. The percentage decrease in eosinophils 24 h after allergen challenge was 15% in the placebo group and 55% in the IL-12 group (table 2).

Discussion

We have shown that systemic IL-12 causes a decrease in blood and sputum eosinophils, but has only minor effects on airway hyper-responsiveness to histamine, and no effect on airway responsiveness to inhaled allergen. This is analogous to the finding that a monoclonal antibody against IL-5 caused profound suppression of blood and sputum eosinophils without apparent effects on airway hyper-responsiveness and the late asthmatic response.[17] Taken together, these studies question the role of eosinophils in being solely responsible for the induced hyper-responsiveness and the late asthmatic response.

During the course of four injections of IL-12, concentrations of blood eosinophils progressively decreased to low normal numbers, suggesting that additional injections could have caused further declines in blood eosinophil numbers. Notably, there were effects on blood eosinophil counts even after the first injection of 0·1 μg/kg, so a course of IL-12 injections at lower doses could be efficacious. The present study did not directly investigate effects of IL-12 on symptoms in patients with asthma, but in those with long-term eosinophilic asthma, it is possible that the decrease in eosinophils to low normal values could still decrease airways remodelling.

The decline in blood and sputum eosinophils was not associated with a significant decrease in airway hyper-responsiveness to histamine. This finding is consistent with animal studies that do not show airway hyper-responsiveness in relation to eosinophilic inflammation,[18] including the observations in rats in which ICAM-1 antibody can inhibit the response without decreasing eosinophilic inflammation,[19] although ciclosporin A can inhibit eosinophil influx without affecting airway hyper-responsiveness.[20] In human allergic asthma, dissociation between airway inflammation and the response has also been reported.[21,22]

IL-12 treatment had no apparent effect on the late asthmatic reaction despite causing a striking decline in blood and sputum eosinophils, suggesting that recruitment of eosinophils from the bone marrow into the blood might not be essential to mount the late response. Indeed, redundancy could be present, and the late asthmatic reaction may be caused by effects of T cells, mast cells, eosinophils, and other inflammatory cells.[23,24] Alternatively, after treatment with IL-12 there could be sufficient residual eosinophils present in the airways to still allow the reaction to occur. Another possibility is that the doses of IL-12 might have been too low to see substantial effects; these effects being limited by the toxic effects of higher doses.

At the doses used in our study, IL-12 caused too many adverse events to justify its clinical use in mild disease. However, this disadvantage might be circumvented by topical application or giving lower doses. In early clinical trials with IL-12, there were two deaths from multiple organ-system failure in patients with advanced malignancies.[25] After the introduction of gradual incremental dose increases, IL-12 has been used to safely treat several hundred patients. Nevertheless, in most patients receiving IL-12 at the dose regimen used, a flu-like syndrome and transient increases in serum hepatic transaminases occurred. An additional safety concern is that cardiac arrhythmias can be precipitated in patients with a previous history of palpitations and cardiac disease.

On the basis of animal studies, IL-12 has the potential to mediate disease modification and exert long-term effects on the natural history of asthma.[26] The effects on blood and sputum eosinophils noted in this report do not provide evidence for disease modification, because blood eosinophil counts began to increase 6 days after each injection, and eosinophil numbers returned to baseline values at 4 weeks after the fourth injection. IL-12 may have to be given at the same time as sensitisation—eg, in early childhood to suppress subsequent allergic responses, since T cells may already be committed to a Th2 type response if already sensitised. However, studies in childhood have major safety and ethical implications. IL-12 is potentially best used as an adjuvant[27] in conjunction with allergen immunotherapy, or in the context of seasonal exposure to grass pollen.

Contributors

Jamey Khan and Varsha Kanabar undertook sputum processing and analysis of sputum data. Juergen Bock did the statistical analyses. All other authors were involved in the design of the study, carrying out clinical procedures, and writing the manuscript. Peter Barnes was the principal investigator.

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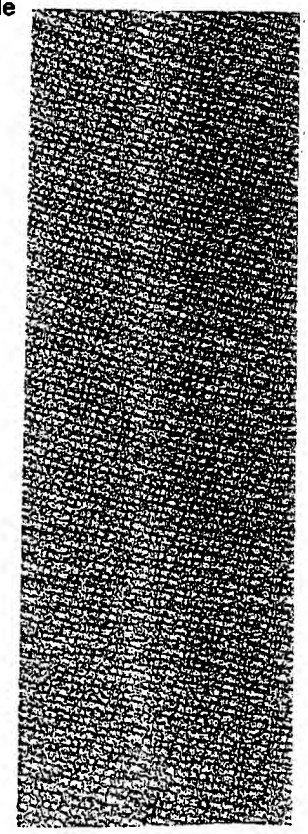

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